CLAIMS

1. A chimeric binding protein that is immunogenic in an animal, said chimeric binding protein being one that binds specifically to a first receptor, said first receptor being one that binds a second receptor present in an antigen of said animal, wherein said chimeric binding protein comprises:
- a B-cell epitope in the form of a binding site that specifically binds the first receptor and which competes with the second receptor for binding to the first receptor,
- a scaffold protein structure that stabilises the 3D conformation of the binding site, said scaffold protein structure being autologous in said mammal, and
- at least one tolerance breaking amino acid sequence, which is heterologous in said animal and which binds to an MHC Class II molecule in said animal.

2. The chimeric binding protein according to claim 1, wherein said scaffold protein structure is derived from an abundant protein, preferably an abundant serum protein.

3. The chimeric binding protein according to claim 1 or 2 wherein said scaffold protein structure is derived from albumin, an immunoglobulin, transferrin, and α_2-macroglobulin.

4. The chimeric binding protein of any one of claims 1-3, wherein said scaffold protein structure is derived from IgG.

5. The chimeric binding protein of any one of claims 1-4, wherein said scaffold protein structure is derived from the non-idiotypic region of a molecule selected from the group consisting of a complete antibody and a fragment thereof such as an F(ab')2 fragment, an Fab fragment, and an scFv.

6. The chimeric binding protein of any one of claims 1-5, wherein said scaffold protein structure comprises a substantially complete amino acid sequence of a polypeptide autologous in said animal.

7. The chimeric binding protein of any one of claims 1-6, wherein said scaffold protein structure comprises a substantial number of B-cell epitopes found in the autologous scaffold protein structure in the animal.

8. The chimeric binding protein of any one of claims 1-7, wherein said scaffold protein structure has substantially the same tertiary structure of a polypeptide autologous in said animal.

9. The chimeric binding protein of any one of claims 1-8, wherein said B-cell epitope is constituted by the idiotype of an antibody.

10. The chimeric binding protein of any one of claims 1-9, wherein said first receptor is the idiotype of an antibody or a specific binding region of a ligand that binds the second receptor in said animal.

11. The chimeric binding protein of any one of claims 1-10, wherein said first receptor is the idiotype of a monoclonal antibody.

12. The chimeric binding protein of any one of claims 1-11, wherein said tolerance breaking amino acid sequence is introduced by means of amino acid insertion or substitution in the amino acid sequence of the scaffold protein structure.

13. The chimeric binding protein of any one of claims 1-12, wherein the animal is a human being.

14. The chimeric binding protein of any one of claims 1-13, which is an anti-idiotypic antibody or an effectively binding fragment thereof that is modified so as to include said tolerance breaking amino acid sequence.

15. The chimeric binding protein of any one of claims 1-14, wherein the antigen of said animal that includes said second receptor is selected from the group consisting of immunoglobulin E, CD20, CD11a, beta amyloid, HER-2, and TNFα.

16. The chimeric binding protein of any one of claims 1-15, which further comprises
- at least one first moiety which effects targeting of the chimeric binding protein to an antigen presenting cell (APC) or a B-lymphocyte, and/or
- at least one second moiety which stimulates the immune system, and/or
- at least one third moiety which optimises presentation of the chimeric binding protein to the immune system.

17. The chimeric binding protein according to claim 16, wherein the tolerance breaking amino acid sequence and/or the first and/or the second and/or the third moiety is/are present in the chimeric binding protein by being bound to suitable side groups in the scaffold protein structure.

18. The chimeric binding protein according to claim 17, wherein the tolerance breaking amino acid sequence and/or the first and/or the second and/or the third moiety is/are present in the scaffold protein structure by means of at least one amino acid substitution and/or deletion and/or insertion and/or addition.

19. The chimeric binding protein according to any one of claims 1-18, wherein the tolerance breaking amino acid sequence is promiscuous in the animal species to which said animal belongs.

20. The chimeric binding protein according to any one of claims 1-19, wherein the tolerance breading amino acid sequence is selected from a natural promiscuous T helper cell epitope and an artificial MHC-II binding peptide sequence.

21. The chimeric binding protein according to claim 20, wherein the natural T-cell epitope is selected from a Tetanus toxoid epitope such as P2 or P30, a diphtheria toxoid epitope, an influenza virus hemagluttinin epitope, and a P. falciparum CS epitope, and wherein the artificial MHC-II binding peptide sequence is a PADRE peptide.

22. The chimeric binding protein according to any one of claims 16-21, wherein the first moiety is a substantially specific binding partner for a B-lymphocyte specific surface antigen or for an APC specific surface antigen, such as a hapten or a carbohydrate for which there is a receptor on the B-lymphocyte or the APC.

23. The chimeric binding protein according to any one of claims 16-22, wherein the second moiety is selected from a cytokine and a heat-shock protein.

24. The chimeric binding protein according to claim 23, wherein the cytokine is selected from, or is an effective part of, interferon γ (IFN-γ), Flt3L, interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 12 (IL-12), interleukin 13 (IL-13), interleukin 15 (IL-15), and granulocyte-macrophage colony stimulating factor (GM-CSF), and the heat-shock protein is selected from, or is an effective part of any of, HSP70, HSP90, HSC70, GRP94, and calreticulin (CRT).

25. The chimeric binding protein according to any one of claims 16-24, wherein the third moiety is of lipid nature, such as a palmitoyl group, a myristyl group, a farnesyl group, a geranyl-geranyl group, a GPI-anchor, and an N-acyl diglyceride group.

26. A nucleic acid fragment that encodes the chimeric binding protein according to any one of claims 1-25, or a nucleic acid fragment complementary thereto.

27. A vector carrying the nucleic acid fragment according to claim 26, such as a vector that is capable of autonomous replication.

28. The vector according to claim 27, which is selected from the group consisting of a plasmid, a phage, a cosmid, a mini-chromosome, and a virus.

29. The vector according to claim 27 or 28, comprising, in the 5'→3' direction and in operable linkage, a promoter for driving expression of the nucleic acid fragment according to claim 26, optionally a nucleic acid sequence encoding a leader peptide enabling secretion of or integration into the membrane of the polypeptide fragment, the nucleic acid fragment according to claim 26, and optionally a terminator.

30. The vector according to any one of claims 27-29 which, when introduced into a host cell, is capable or incapable of being integrated in the host cell genome.

31. The vector according to any one of claims 27-30, wherein a promoter drives expression in a eukaryotic cell and/or in a prokaryotic cell.

32. A transformed cell carrying the vector of any one of claims 27-30, such as a transformed cell which is capable of replicating the nucleic acid fragment according to claim 26.

33. The transformed cell according to claim 32, which is a microorganism selected from a bacterium, a yeast, a protozoan, or a cell derived from a multicellular organism selected from a fungus, an insect cell such as an S2 or an SF cell, a plant cell, and a mammalian cell.

34. The transformed cell according to claim 32-33, which expresses the nucleic acid fragment defined in claim 26, such as a transformed cell, which secretes or carries on its surface, the chimeric binding protein defined in any one of claims 1-25.

35. A composition for inducing production of antibodies against an antigen in the autologous host, the composition comprising

- a chimeric binding protein according to any one of claims 1-25, and
- a pharmaceutically and immunologically acceptable carrier and/or vehicle and/or adjuvant

36. A composition for inducing production of antibodies against an antigen in the autologous host, the composition comprising
- a nucleic acid fragment according to claim 26 or a vector according to any one of claims 27-31, and
- a pharmaceutically and immunologically acceptable carrier and/or vehicle and/or adjuvant.

37. A stable cell line which carries the vector according to any one of claims 27-31 and which expresses the nucleic acid fragment according to claim 26, and which optionally secretes or carries the chimeric binding protein according to any one of claims 1-25 on its surface.

38. A method for the preparation of the cell according to any one of claims 32-34, the method comprising transforming a host cell with the nucleic acid fragment according to claim 26 or with the vector according to any one of claims 27-31.

39. A method for preparing the chimeric binding protein of any one of claims 1-25, the method comprising the following steps:
1) providing a first molecule, which binds to a self-antigen of interest in an animal and which includes the first receptor,
2) immunizing, with the first molecule optionally being coupled to an immunogenic carrier, a transgenic animal that produces antibodies that are autologous in the animal harbouring the self-antigen or that are autologous in the animal harbouring the self-antigen except for the fact that they also include at least one amino acid sequence that breaks tolerance in the animal,
3) preparing and isolating hybridomas that produce antibodies that bind the first molecule,
4) screening the hybridomas of step 3 for their ability to produce antibodies that selectively bind to said first receptor, and
5) transforming a suitable host cell with at least genetic material that encodes antibodies or functional parts thereof where the genetic material is or can be isolated from the hybridomas of step 4 that produce selectively binding antibodies,
6) culturing the host cells transformed in step 5 under conditions that facilitate production of at least the antibodies or functional fragments thereof, and recovering the antibodies or functional fragments thereof from the host cell culture.

40. A method for preparing the chimeric binding protein of any one of claims 1-25, the method comprising the following steps:
1) providing a first molecule, which binds to a self-antigen of interest in an animal and which includes the first receptor,
2) screening a library of second molecules for their ability to selectively bind to said first receptor of said first molecule,

3) isolating the members of the library that selectively binds in step 2, and
4) preparing, by means of synthesis or recombinant technology, the chimeric binding protein that contains at least a) the binding site of a member isolated in step 3, b) a scaffold protein structure autologous in the animal that stabilises the native 3D structure of said binding site, and c) a non-human MHC Class II binding amino acid sequence; or
i) preparing, by means of synthesis or recombinant technology, a chimeric binding protein containing 1) the second receptor or a mimotope thereof in correct, native 3D conformation, 2) a scaffold protein structure autologous in the animal, said scaffold protein structure stabilising said 3D conformation and being derived from another molecule in the animal than the second receptor, and 3) the tolerance breaking amino acid sequence.

41. The method according to claim 39 or 40, wherein the first molecule is an antibody, preferably a monoclonal antibody.

42. The method according to claim 41, wherein the first receptor is the idiotype of the antibody.

43. The method according to any one of claims 39-42, wherein the screening in step 3 includes an exclusion step that allows identification of members of the library that bind the first molecule outside the first receptor so as to exclude such members from subsequent steps.

44. The method according to claim 43, wherein said exclusion step involves
a) a test of the library members' ability to bind to the parts of the first molecule that are outside the first receptor, so as to allow exclusion of library members that exhibit such binding, and/or
b) a test of the library members' ability to compete with the second receptor for binding to the first receptor that allows exclusion of library members that do not exhibit such ability.

45. The method according to any one of claims 40-44, insofar as these are dependent on claim 40, wherein step 3 involves phage display of the second molecules.

46. The method according to any one of claims 40-44, insofar as these are dependent on claim 40, wherein step 3 involves that the library of second molecules is subjected to ribosome display, mRNA-display, or yeast surface display.

47. A method for down-regulating a self-antigen or a cell that displays epitopes of said self-antigen in an animal, the method comprising presenting the animal's immune system with an immunogenically effective amount of a chimeric binding protein according to any one of

claims 1-25 so as to induce a specific immune response against the self-antigen that includes in its structure the second receptor defined in claim 1 or 25.

48. The method according to claim 47, wherein an effective amount of the chimeric binding protein is administered to the animal via a route selected from the parenteral route such as the intracutaneous, the subcutaneous, and the intramuscular routes; the peritoneal route; the oral route; the buccal route; the sublinqual route; the epidural route; the spinal route; the anal route; and the intracranial route.

49. The method according to claim 48, wherein the effective amount is between 0.5 µg and 2,000 µg of the chimeric binding protein.

50. The method according to any one of claim 47-49, wherein the chimeric binding protein is contained in a virtual lymph node (VLN) device.

51. The method according to any one of claims 47-50, wherein the chimeric binding protein has been formulated with an adjuvant which facilitates breaking of autotolerance to autoantigens.

52. The method according to claim 47, wherein presentation of the chimeric binding protein to the immune system is effected by introducing nucleic acid(s) encoding the chimeric binding protein into the animal's cells and thereby obtaining in vivo expression by the cells of the nucleic acid(s) introduced.

53. The method according to claim 52, wherein the nucleic acid(s) introduced is/are selected from naked DNA, DNA formulated with charged or uncharged lipids, DNA formulated in liposomes, DNA included in a viral vector, DNA formulated with a transfection-facilitating protein or polypeptide, DNA formulated with a targeting protein or polypeptide, DNA formulated with Calcium precipitating agents, DNA coupled to an inert carrier molecule, DNA encapsulated in chitin or chitosan, and DNA formulated with an adjuvant.

54. The method according to claim 53, wherein the nucleic acid(s) is/are contained in a VLN device.

55. The method according to any one of claims 47-54, which includes at least one administration/introduction per year, such as at least 2, at least 3, at least 4, at least 6, and at least 12 administrations/introductions.

56. The method according to claim 47, wherein presentation to the immune system is effected by administering a non-pathogenic microorganism or virus which is carrying and expressing a nucleic acid fragment according to claim 26.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 15453PCT00	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/DK 03/00859	International filing date *(day/month/year)* 11.12.2003	Priority date *(day/month/year)* 11.12.2002
International Patent Classification (IPC) or both national classification and IPC C07K14/705		
Applicant PHARMEXA A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.06.2004	15.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer van Heusden, M Telephone No. +49 89 2399-8145

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK 03/00859

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-50 as originally filed

Claims, Numbers

1-51 received on 04.11.2004 with letter of 04.11.2004

Drawings, Sheets

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. partially 1-51

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. partially 1-51 are so unclear that no meaningful opinion could be formed *(specify)*:

   see separate sheet

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. partially 1-51

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	7,8,10-13,17-51
	No:	Claims	1-6, 9, 14-16
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-51
Industrial applicability (IA)	Yes:	Claims	1-41
	No:	Claims	42-51 (?)

2. Citations and explanations

see separate sheet

Additional remarks to section V:

1. Citations

1.1 The documents mentioned in this report are numbered as in the International Search Report (ISR), i.e. D1 corresponds to the first document of the ISR etc.

2. Novelty and Inventive step (Article 33(2)(3) PCT)

2.1 The present application relates to a chimeric binding protein. The subject matter of amended claim 1 is now limited to a chimeric binding protein that comprises a B cell epitope specifically binding a first antibody or 'a region of a ligand which specifically binds a receptor', a human immunoglobulin-derived scaffold structure and a heterologous tolerance breaking amino acid sequence.
The IPEA maintains that the binding protein is defined largely by functional features: the B-cell epitope is not defined by structural features but by the functional feature of binding the idiotype of a (any) antibody or a (any) ligand to a (any) receptor in a (any) human antigen. The scaffold protein is defined as being derived from a human immunoglobulin (=product by process) and can thus be any part of or even any modification of a human Ig. The scaffold protein structure is thus also not structurally defined. Also the tolerance breaking amino acid sequence is defined by being heterologous in human (negative definition) and by a result to be achieved: binding to an MHC Class II molecule in a human. As a result the IPEA maintains that the chimeric binding protein according to claim 1 lacks clarity.

2.2 It appears from the description that an anti-idiotypic antibody comprising a T-cell epitope falls within the scope of claim 1.
Document D10 discloses protein conjugates (fusion proteins) comprising a T-cell antigen (=tolerance breaking amino acid sequence) which is capable of forming a complex with MHC class I or II molecules (p. 3, §3) and further comprising a binding partner. Said binding partner is an anti-idiotypic antibody (p. 5, last § and p. 8, last §). D10 further discloses that the T-cell antigen can be a P2 or P30 tetanus toxin epitope or Plasmodium epitopes (p. 7, first §).
Whether or not the anti-idiotypic antibody disclosed in D10 is 'derived from a human antibody' is not disclosed. But due to the wording 'derived from' in claim 1, the structural features of the Ig scaffold protein are undefined. Thus the anti-

idiotypic antibody disclosed in D10 falls within the scope of claim 1.
Furthermore the applicant has argued that autoimmune B-cell lymphoma is a very rare condition. This is irrelevant with respect to the fact that the anti-idiotypic antibody as disclosed in D10 falls within the scope of claim 1.
The applicant has also argued that D10 relates to a different concept, namely to target cytotoxic T-cells to those cells that bind the conjugates according to D10. What the conjugates are used for in D10 is irrelevant with respect to the fact that the conjugates themselves are disclosed and anticipate the chimeric binding protein of claim 1. Said argument is relevant only for those claims relating to the use of the chimeric binding protein (claims 30, 31 and 42-51) which are novel over D10.

Therefore document D10 anticipates the subject matter of claims 1-6, 9 and 14-16.

2.3 The subject matter of claim 34 relates to a method defined by steps 1) to 6). Step 2 relates to immunizing a transgenic animal. Said transgenic animal may produce an antibody autologous in human **OR** a human autologous antibody that also includes a tolerance breaking sequence (the latter part is optional!). Thus said six steps correspond to a conventional method of making anti-idiotypic antibodies by immunization (the inclusion of a tolerance breaking sequence being optional), which is obviously not novel (see e.g. document D7). Claim 35 differs from claim 34 in that binding molecules are obtained by screening a library of second molecules, rather than by immunizing. Screening libraries for binding molecules is a routine technique in the art (see e.g. D7: Fab phage display library).

2.4 Claims 7, 8, 10-13, 17-20 do not appear to include any additional matter that could render them inventive as such. The subject matter of claim 8 differs from the disclosure in D10 in that the T-cell antigen is not (covalently) coupled to the binding partner, but is inserted or substituted into the protein scaffold structure. This is merely an alternative method of fusing the two components and can be achieved using conventional recombinant DNA technology.
Also the subject matter of claims 21-29 and 32-41 does not add any matter that could render them inventive as such. Thus they would be allowable only in combination with a novel and inventive main claim.

2.5 With respect to the subject matter of claims 30, 31 and 42-51, relating to the use of the chimeric binding protein in vaccination, said subject matter has not been suggested in the cited prior art. However, in view of the well known difficulties in therapeutic vaccination, in combination with the absolute absence of any experimental evidence in the present application, the IPEA has serious doubts as to whether the chimeric binding protein according to claim 1 can indeed be used successfully in therapeutic vaccination and whether its administration results in down-regulation of a self-antigen. It seems that the present application relates to a novel hypothesis for the provision of a therapeutic vaccine, which hypothesis, however, has not been tested at all. In other words the application does not provide any evidence that the problem posed by the invention has been solved. Therefore inventive step cannot be acknowledged.
The applicant is already informed that in the case of a European application, an objection for lack of enablement will be raised.

3. Industrial applicability (Article 33(4) PCT)

3.1 The subject matter of claims 1-41 appears to be industrially applicable.

3.2 The subject matter of claims 42-51 includes methods of treatment of the human or animal body and is thus excluded from examination by Article 34(4)(a)(I) PCT in combination with Rule 67(iv) PCT. For the assessment of these claims on the question whether they are industrially applicable, no unified criteria exist in PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment. The applicant is already informed that in the case of a European application, claims 47-56 do not seem to be allowable because 'methods of treatment of human or animal body by surgery or by therapy and diagnostic methods practised on the human or animal body shall not be regarded as inventions which are susceptible of industrial application'.